Indian Journal of Science and Technology http://www.indjst.org Vol.1 No 4 (Sep. 2008)

Effect of NaCl on biochemical changes and endophytic fungal assemblages in the leaves of a mangrove, *Ceriops roxburghiana* Arn.

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Abstract: One of the universal protective responses of plants to any type of stress is the accumulation of low-molecular organic substances such as amino acids, sugars and proteins. In this study, the effect of salinity on leaf pigments, proteins, free amino acids, proline, polyphenols, sugars and starch content was investigated. Status on endophytic fungal assemblages in the salt treated leaves of a mangrove, *C. roxburghiana* was studied under hydroponics pot culture. The photosynthetic pigments, sugars and protein concentrations of leaves were reduced by salinity and that the effect was aggravated by the long duration of salinity. Total contents of both chlorophyll and carotenoids decreased significantly by the treatment of NaCl. Total amino acid pool decreased by salinity, but the proline content increased only marginally. In the control, proline level did not change during the entire period of the study. The significant increase of accumulation of proline in leaves is implicated in osmotic adjustment to salinity. The endophytic fungal assemblage was also investigated. Among the 7 species, isolated *Phyllosticta* sp. was found frequently more when compared to other fungi. The results show that the endophytic fungi like *Pestalotiopsis* sp. and *Phyllosicta* sp. can be sustained and regenerated at low salinity condition in halophytes.

Keywords: *Ceriops roxburghiana*, Salinity, Biochemical changes, Endophytic fungi.

Introduction

A pot experiment was carried out under glasshouse conditions to investigate the effects of salt induced biochemical changes in a salt secretor mangrove, *Ceriops roxburghiana*, Arn. These species are typical woody mangrove shrubs with opposite leaves belonging to the family Rhizophoraceae. They were found growing in the tidal forests of mangrove belt of Pichavaram on the north east coast of Tamil Nadu, India, about 10 Km away from Annamalai University Campus (11° 24' N and 79° 44' E). Salinity is a major abiotic stress reducing the yield of a wide variety of crops all over the world (Tester & Davenport, 2003). Salinity, in particular, is an increasing problem affecting 20% of the world's cultivated land and nearly half of the area under irrigation, to the point that genetic improvement of salt tolerance has become an urgent need for the future of agriculture in arid and semi-arid regions (Boyer, 1982). Breeding of salt resistant crop varieties will require a clear understanding of the complex mechanisms of salt stress tolerance, which we are still lacking despite intensive research during the last decade (Apse & Blumwald, 2002).

Although the level of salts in most irrigation waters is below the threshold for the more sensitive crops, salt accumulation can reduce growth and yield of even the more tolerant crops. Overcoming salt stress is a main issue in these regions to ensure agricultural sustainability and continued food production (Heuer, 2003). Plants need to maintain internal water potential below that of soil to maintain turgor and sufficient water for growth. This requires an increase in osmotica, either by uptake of solutes from the soil or by synthesis of metabolically compatible solutes (Tester & Davenport, 2003). Compatible solutes, such as proline, are known to accumulate under conditions of environmental stresses to play a role in the process of osmotic adjustment in many crops. Their main role is probably to protect plant cells against the ravages of salt by preserving the osmotic balance, stabilizing sub-cellular structures, such as membranes and proteins, and scavenging reactive oxygen species. Therefore, exogenous application of these compounds has been suggested as an alternative or additional approach to genetic engineering to improve crop productivity under stress condition (Makale *et al*., 1996). Investigations have demonstrated that a number of ecological factors such as geographic location (Fisher *et al*., 1994; Collado *et al*., 1999), differences in site (Okane *et al*., 1997) and microclimate (Johnson & Whitney 1989), anthropological modifications (Sieber, 1989), age and specificity of the colonized tissue (Bills & Polishook, 1991; Sahashi *et al*., 2000) could greatly influence endophytic assemblages as well as their metabolic activities. Thus, the endophytic fungi are expected to be a potential source for new natural bioactive agents. In recent years, the quest for the isolation of new compounds from medicinal plants has become a fascinating area of research. In the present investigation, the effect of NaCl on

Indian Journal of Science and Technology http://www.indjst.org Vol.1 No 4 (Sep. 2008)

pigments, proteins, free amino acids, proline, polyphenols, sugars, starch content and endophytic fungal assemblage in *C. roxburghiana* under hydroponic culture were studied with an aim to obtain insights into the changes in osmotic composition associated with salt accumulation.

Materials and methods

Plant materials and culture conditions

In the present investigation, seedlings of *C. roxburghiana* were collected. About 750, uniform sized, one month old healthy seedlings were collected from the tidal mangrove forest soil without damaging the root system. These seedlings were washed thoroughly and brought to the Botanical garden of Annamalai University. Polythene sleeves (7" X 5") were filled with homogenous mixture of garden soil comprising red earth, sand and farm-yard manure (1:2:1). The selected healthy seedlings were transferred to polythene sleeves. These plants were irrigated with tap water and allowed to establish well. The seedlings were fully established in the polythene sleeves within a month and they were transferred to experimental site and covered with a transparent polythene sheet to protect them from rain water.

Salt treatment

The preliminary experiments were carried out in seedlings of *C. roxburghiana* raised in the greenhouse treated with different concentrations of NaCl (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mM) in order to determine the viable range of salinities. The control was maintained without NaCl treatment. The seedlings treated with 700 mM NaCl and above could not survive a week while at 600 mM NaCl the plant can survived for more than 30 days. Therefore, 600 mM NaCl was chosen as suitable concentration for further investigation. Leaf samples were collected periodically at two months intervals for six months after salt treatment and the biochemical changes were studied. The endophytic fungal assemblages in the leaves were also investigated.

Extraction and Estimation of pigments

Prior to extraction, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Chlorophyll extraction was carried out on fresh leaf. About 0.5 g of leaves was homogenized with 80% cold acetone in a pre-chilled mortar and pestle in dark at 4°C. The homogenate was centrifuged at 8000 x g for 10 min. The supernatant was collected and the absorption spectra were recorded at 663 and 645 nm using DU-40 Spectrophotometer. Estimation of Chl a, Chl b and total Chl were followed by the method of Arnon (1949). The chlorophyll content was calculated by using the formula:

Total chlorophyll (mg/ml) =
$(0.0202) \times (O.D.\ 645) + (0.00802) \times (O.D.\ 663)$

Chlorophyll 'a' (mg/ml) =
$(0.0127) \times (O.D.\ 663) - (0.00269) \times (O.D.\ 645)$

Chlorophyll 'b' (mg/ml) =
$(0.0229) \times (O.D.\ 645) - (0.00468) \times (O.D.\ 663)$

Estimation of Carotenoids

Total carotenoids were calculated according to the method of Arnon (1949). Aqueous acetone extracts were shaken three times with an equal volume of hexane in a separating funnel and the combined hexane fractions were washed with equal volume of water to separate caroteins from xanthophylls. The hexane fraction containing the carotenoid was extracted repeatedly with 90 per cent methanol. The hexane fraction containing caroteins and methanol fraction containing xanthophylls was measured by utilizing the values of absorbance at 424 nm and 480 nm respectively.

Total carotenoids (µg/ml) =
$A\ 480 + (0.114 \times A - 0.638 \times A.\ 645)$

Extraction and Estimation of total free amino acids

Total free amino acids were extracted with 70% Ethanol and estimated by the method described by Moore and Stein (1948) using Ninhydrin reagent. The absorbance was recorded at 570 nm in a DU-40 Spectrophotometer and the total free amino acids were calculated from the standard graph prepared using glycine (0-100 µg).

Extraction and Estimation of total leaf protein

Total leaf protein was extracted and estimated by the method of Lowry *et al*. (1951). Bovine Serum Albumin (BSA) was used as standard protein.

Estimation of total sugars and starch

Alkaline copper method (Nelson,1944; Somogyi,1945) was followed for estimation of reducing sugars using arsenomolybdate reagent. Absorbance was recorded at 510 nm. Reducing sugar content was determined from a standard curve prepared against glucose (0-50 µg).

Total soluble sugars and starch were estimated by anthrone-sulphuric acid method of McCready *et al*. (1950) using 0.2% anthrone in concentrated H_2SO_4 as reagent. Absorbance was recorded at 630 nm. Standard curve was plotted with 0-100 µg of glucose. The starch concentration was determined by multiplying the obtained value by 0.9 with glucose.

Extraction and Estimation of Proline

Proline content was extracted from the leaf tissues by the method described by Bates *et al.* (1973). Approximately, 0.5 g of fresh leaf material

was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and filtered through Whatman's No.2 filter paper. Two milliliter of the filtrate was mixed with 2 ml of acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with a DU-40 Spectrophotometer. L-proline (Sigma) was used as standard.

Isolation of endophytic fungi

The effect of NaCl treatment on the endophytic fungal assemblage was also studied. The NaCl treated leaf samples were collected and surface sterilized by a method of modified Dobranic *et al*. (1995). The leaves were thoroughly washed in running tap water and about one hundred and fifty small pieces of approximately 0.5 cm diameter were cut from the upper, middle and lower portion with the aid of a flame-sterilized cork borer. Then the leaf discs were surface sterilized by immersion in 70% ethanol for 5 seconds, followed by 4% sodium hypochlorite for 90 seconds and then rinsed in sterile distilled water for 10 seconds. The excess moisture was blotted in a sterile filter paper. The surface sterilized leaf segments were evenly spaced in petri dishes containing potato dextrose agar (PDA) medium (amended with chloramphenicol 150 mgl^{-1}). The petri dishes were sealed using Parafilm^{TM} and incubated at 26 ± 1° C in a light chamber (Bills & Polishook, 1992). The petri dishes were monitored every day for the growth of endophytic fungal colonies from the leaf segments. The hyphal tips, which grew out from leaf segments were isolated and brought into pure culture. The isolated endophytic fungi were identified down to species level using standard monographs.

Colonization Frequency (CF %)

The Colonization Frequency (CF %) of a single endophyte species in the leaf tissue was calculated by using the following formula (Hata & Futai, 1995).

$$CF\ \% = \frac{\text{The No: of segments colonized by an endophyte}}{\text{Total number of segments}} \times 100$$

Statistical Analysis

The results obtained for the biochemical changes were statistically analyzed using ANOVA.

Results and discussion

Plants were more resistant to salinity at the initial stage but more sensitive with the increasing duration. The effects of salinity on leaf pigments, proteins, free amino acids, proline, polyphenols, sugars, starch content and endophytic fungal assemblage in *C. roxburghiana* were investigated. The mangrove, *C. roxburghiana* could tolerated maximum NaCl up to 600 mM concentration and could be maintained for more than 30 days. The results obtained showed that high salinity reduced chlorophyll, sugar, protein and amino acid content. However, it increased starch and proline content.

Effect of salinity on biochemical changes in leaves

Changes in the photosynthetic pigments: Total contents of both chlorophyll and carotenoids were decreased significantly by the treatment of NaCl in *C. roxburghiana*. The total chl; chl a : b ratio was expressed on unit fresh weight and decreased at 400 mM NaCl treatment as compared to control at 60 days and 120 days (Table 1) respectively. A similar trend was observed in carotenoids content, expressed in fresh weight basis. At high salt concentration (400 mM), a decrease in the photosynthetic pigments was observed but only marginally during the period of observations. Yeo

Table 1. Effects of NaCl on Chl a, Chl b, total chlorophyll, Chl a\b ratio and carotenoid content in leaves of C. roxburghiana

Period of treatment (days)	NaCl Conc. (mM)	Chl *a* (mg g^{-1} fresh wt.)	Chl *b* (mg g^{-1} fresh wt.)	Total Chl (mg g^{-1} fresh wt.)	Chl *a/b*	Carotenoid (mg g^{-1} fresh wt.)
0	0	0.425±0.0051^{a}	0.183±0.0028^{a}	0.586±0.0045^{a}	1.987±0.01^{e}	0.112±0.000^{a}
60	0	0.452±0.0069^{a}	0.198±0.0030^{a}	0.650±0.0092^{a}	2.282±0.03^{e}	0.110±0.001^{a}
	400	0.241±0.003^{g}	0.131±0.0020^{f}	0.372±0.0056^{f}	1.839±0.02^{g}	0.056±0.000^{d}
120	0	0.697±0.0106^{a}	0.292±0.0045^{a}	0.989±0.0064^{a}	2.386±0.03^{c}	0.141±0.002^{a}
	400	0.310±0.0047^{e}	0.245±0.0037^{e}	0.550±0.0036^{e}	1.265±0.01^{g}	0.078±0.001^{d}

Different letters besides figures indicate statistically significant at P=0.05.

et al. (1985) also reported that the inhibition of net photosynthesis in rice by salinity is mediated by water deficit in the leaf cells due to accumulation of salt in the apoplast. A decrease in chlorophyll concentration in salinied plants could be attributed to increased activity of the chlorophyll-degrading enzyme chlorophyllase (Reddy & Vora, 1986).

Table 2. Effects of NaCl on total sugar, starch and protein content in leaves of C. roxburghiana

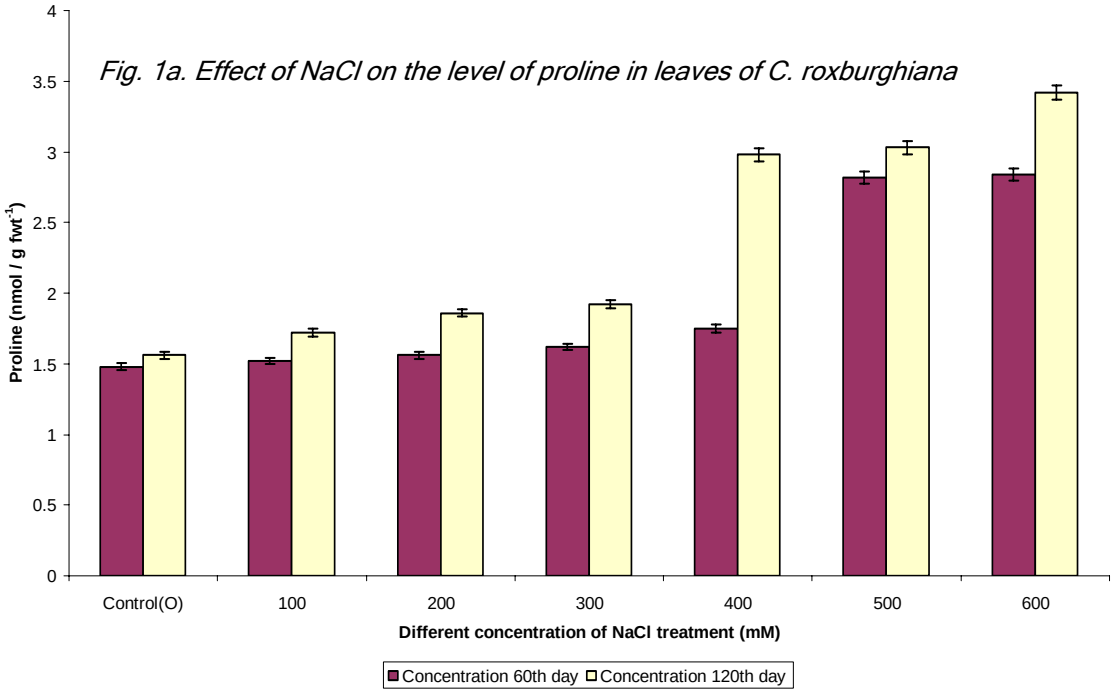
Period of treatment (days)	NaCl (mM)	Total sugar (mg g^{-1} fresh wt.)	Starch (mg g^{-1} fresh wt.)	Protien (mg g^{-1} fresh wt.)
0	0	6.70±0.1981^{b}	5.38±0.1032^{d}	2.58±0.0344 ^{a}
60	0	6.72±0.1344^{b}	5.40±0.1082^{d}	2.68±0.0409^{a}
	400	4.58±0.0916^{a}	6.59±0.1318^{a}	2.16±0.0329^{e}
120	0	7.35±0.147^{b}	6.29±0.1258^{e}	3.29±0.0503^{a}
	400	5.45±0.109^{a}	8.84±0.1768^{d}	2.25±0.0344^{e}

Different letters besides figures indicate statistically significant at P=0.05

Changes in total sugar, starch and protein: Our study shows that photosynthetic pigments, sugars and protein concentrations of leaves were reduced by salinity and that the effect was aggravated by the long duration of salinity. As salinity adversely influenced the photosynthetic process, photosynthetic production (eg. sugar) was inhibited. Changes in total contents of sugar, starch and protein were observed. In control, there is no significant changes in sugar, starch and protein on unit fresh weight basis were observed in 120 days. At higher concentration of NaCl (400 mM), the total sugar content and total leaf protein content decreased significantly during NaCl treatment (Table 2). Kerepesi *et al*. (1998) also found that sugar contents of leaves decreased in tolerant genotypes of wheat under NaCl stress but drought stress increased the water soluble carbohydrate which had linked to stress tolerance. The marginal change in protein content and protein profile in *C. roxburghiana* suggests that NaCl exposure affects protein synthesis or proteolysis minimally in this plant. On the other hand, the starch content increased significantly at higher concentration of NaCl at 120 days. However, Alamgir and Ali (1999) reported that sugar content increases in some genotypes of rice, but also decreases in some genotypes. Under salinity, the starch content in roots of rice plants declines and unchanged in shoots. The contents of reducing and non-reducing sugars, and the activity of sucrose phosphate synthase increase under salinity, whereas starch phosphorylase activity decreases (Dubey & Singh, 1999).

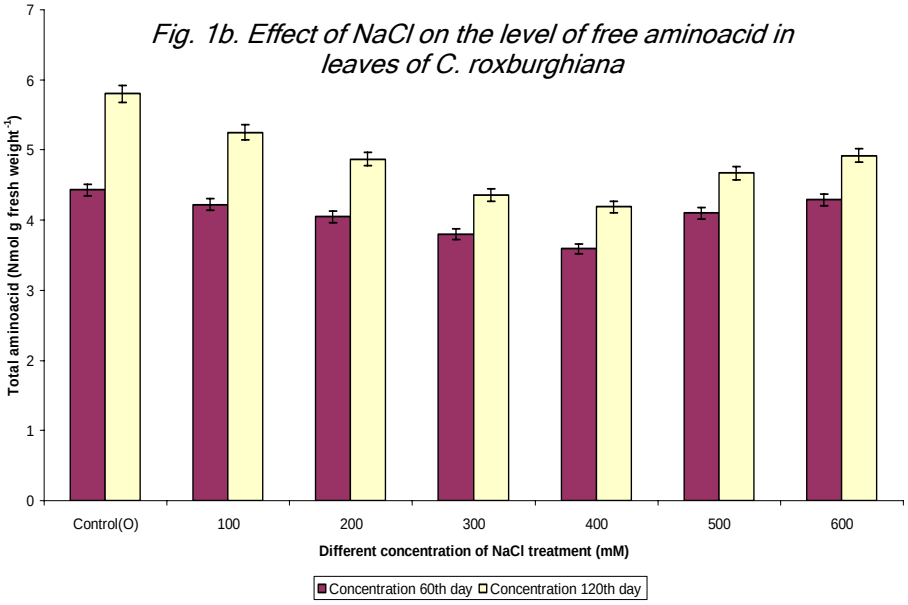
Changes in the level of free amino acid and proline: Proline is also considered to act as a

Fig. 1a. Effect of NaCl on the level of proline in leaves of C. roxburghiana



compatible osmoticum and therefore to be involved in salt resistance (Delauney & Verma, 1993). The effect of NaCl treatment in the level of free amino acid and proline was observed. A significant decreasing trend was observed in total free amino acid content during salt treatment period (Fig. 1a). After 6 days with 400 mM NaCl, the level of free amino acid pool decreased as compared to control. There are no significant changes observed in 400 mM concentration on 6th day when compared to control. Total amino acid pool decreased by salinity in *C. roxburghiana*. Our results contradict with several reports of increase level of free amino acid pool during salt treatment in different plantspecies (Muthukumarasamy *et al*., 2000; Wang & Nil, 2000). Thus, the NaCl treatments on *C. roxburghiana* showed a decrease in total amino acid pool and no change in protein content, which reflect the mode of adjustment to salinity in this salt secreting species.

Fig. 1b. Effect of NaCl on the level of free aminoacid in leaves of C. roxburghiana

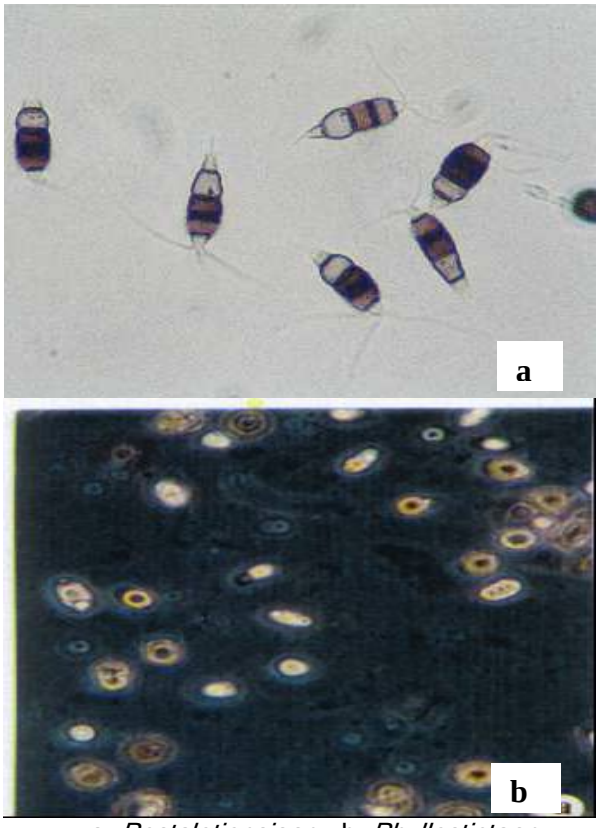


Proline accumulation is a general phenomenon in halophytes. As *C. roxburghiana* is an obligate halophyte and a salt secreting species, it is of interest to study proline accumulation in response to salinity in this plant. It is well known that proline content in leaves of many plants gets enhanced by several stresses including salt stress (Lee & Liu, 1999; Hernandez *et al*., 2000). The proline content increased only marginally, but was found significantly increased at 120th day (Fig. 1b). In the control, proline level did not change during the entire period of the study. The significant increase of accumulation of proline in leaves is implicated in osmotic adjustment to salinity.

Endophytic fungal assemblage

Endophytic fungi were isolated from the leaves of *C. roxburghiana* treated with different concentration of NaCl viz., 100, 200, 300, 400, 500 and 600 mM. A total of 7 species of mitosporic fungi belonging to 6 genera including 3 hyphomycetes and 4 coelomycetes were isolated as endophytes. The Colonization Frequency of fungal endophytes increased significantly at 200 mM concentration and then decreased (Table 3). No growth of endophytic fungi was observed at 400 – 600 mM concentration. Only 2 species i.e. *Pestalotiopsis*

Fig. 2. Conidia of endophytic fungi



a. *Pestalotiopsis* sp. b. *Phyllosticta* sp

Table 3. Colonization Frequency (CF%) of endophytic fungi isolated from salt treated leaves of C. roxburghiana

Endophytic fungi	NaCl concentration (mM)						
	Control	100	200	300	400	500	600
HYPHOMYCETES							
Aspergillus flavus	5	6.666	5	-	-	-	-
Aspergillus fumigatus	8.33	6.666	3.333	-	-	-	-
Fusarium sp.	-	5	1.66	-	-	-	-
COELOMYCETES							
Colletotrichum sp.	1.66	-	3.333	-	-	-	-
Pestalotiopsis sp.	5	8.33	3.333	3.333	-	-	-
Macrophomina sp.	6.666	5	3.333	-	-	-	-
Phyllosticta sp.	18.33	25	21.66	11.66	-	-	-

sp. and *Phyllosticta* sp. were isolated at 300 mM concentration. The endophytic fungi colonized more in 100 mM concentration than control. Among the 7 species, *Phyllosticta* sp. was found to colonize more leas and followed by *Pestalotiopsis* sp (Fig. 2). The results show that the endophytic fungi such as *Pestalotiopsis* sp. and *Phyllosticta* sp. can be sustained and regenerated at low salinity condition in halophytes.

References

1. Alamgir ANM and Ali MY (1999) Effect of salinity on leaf pigments, sugar and protein concentrations and chloroplast ATPase activity of rice (*Oryza sativa* L.). *Bangladesh J. Botany* 28, 145-149.
2. Apse MP and Blumwald E (2002) Engineering salt tolerance in plants. *Current Opinion in Biotechnology* 13, 146-150.
3. Arnon DI (1949) Copper enzymes in isolated chloroplasts, polyphenol oxidase in Beta vulgaris. *Plant Physiol.*24, 1-15.
4. Bakes LS, Waldren RP and Teare LD (1973) Rapid determination of free proline for water-stress studies. *Plant Soil*. 39, 205-207.
5. Bills GF and Polishook JD (1991) Microfungi from *Carpinus caroliniana. Can. J. Bot.* 69,1477-1482.
6. Bills GF and Polishook JD (1992) Recovery of endophytic fungi from *Chamaecyparis thyoides. Sydowia* . 44,1-12.
7. Boyer JS (1982) Plant productivity and environment. *Science*. 218, 443-448.
8. Collado J, Platas G, Gonzales I and Pelaéz F (1999) Geographical and seasonal influence on the distribution of fungal endophytes in *Quercus ilex. New Phytologist*. 143, 525-532.
9. Delauney AJ and Verma DP (1993) Proline biosynthesis and osmoregulation in plants. *The Plant Journal*. 4, 215-223.
10. Dobranic JK, Johnson JA and Alikhan QR (1995) Isolation of endophytic fungi from eastern larch (*Larix laricina*) leaves from New Brunswick, Canada. *Can. J. Microbiol*. 41,194-198.
11. Dubey RS and Singh AK (1999) Salinity induces accumulation of soluble sugars and alters the activity of sugar metabolizing enzymes in rice plants. *Biol. Plant*. 42, 233-239.
12. Fisher PJ, Petrini O, Petrini LE and Sutton BC (1994) Fungal endophytes from the leaves and twigs of *Quercus ilex* L. from England, Majorea and Switzerland. *New Phytol*. 127,133-137.
13. Hata K and Futai K (1995) Endophytic fungi associated with healthy pine needles and needles infested by the pine needle gall midge *Thecodiplosis japonensis. Can. J. Botany*. 73,384-390.
14. Hernandez S, Deleu C and Larher F (2000) Proline accumulation by leaf tissues of tomato plants in response to salinity. *Comptes Rendus de L Academie Des Sciences Serie III-Sciences de La Vie-Life Sciences*. 323, 551-557.
15. Heuer B (2003) Influence of exogenous application of proline and glycinebetaine on growth of salt-stressed tomato plants. *Plant Sci*. 165, 693-699.
16. Johnson JA and Whitney NJ (1989) An investigation of needle endophyte colonization patterns with respect to height and compass direction in a single crown of balsam fir (*Abies balsamea*). *Can. J. Botany*. 67,723-725.
17. Kerepesi I, Galiba G and Banyai E (1998) Osmotic and salt stresses induced differential alteration in water-soluble carbohydrate content in wheat seedlings. *J. Agric. Food Chem*. 46, 5347-5354.
18. Lee TM and Liu CH (1999) Correlation of decreases calcium contents with proline accumulation in the marine green macroalga *Ulva fasciata* exposed to elevated NaCl contents in seawater. *J. Exp. Bot*. 50, 1855-1862.
19. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem*. 193, 265-275.
20. Makale P, Peltonen-Sainio P, Jokinen K, Pehu E, Setala H, Hinkkanen R and Somersalo S

(1996) Uptake and translocation of foliar-applied glycinebetaine in crop plants. *Plant Sci*. 121, 221-230.

21. McCready RM, Guggolz J, Silviera V and Owens HS (1950) Determination of starch and amylase in vegetables. *Anal. Chem*. 22, 1156-1158.
22. Moore S and Stein WH (1948) Photometric ninhydrin method for use in chromatography of amino acids. *J. Biol.Chem*. 176, 367-388.
23. Muthukumarasamy M, Gupta SD and Pannerselvam R (2000) Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by triadimefon in NaCl stressed Raphanus sativus L. *Biol. Plant*. 43, 317-320.
24. Nelson N (1944) A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol.Chem.* 153, 375-380.
25. Okane I, Nakagiri A and Ito T (1997) Endophytic fungi in leaves of ericaceous plant. *Can. J. Botany* 76, 657-663.
26. Reddy MP and Vora AB (1986) Changes in pigment composition, hill reaction activity and saccharides metabolism in bajra (*Pennisetum typhoides* S&H) leaves under NaCl salinity. *Photosynthica*, 20, 50-55.
27. Sahashi NY, Miyasawa T, Kubano S and Ito T (2000) Colonization of beech leaves by two endophytic fungi in northern Japan. *Forest Pathology*. 30,77-86.
28. Sieber TN (1989) Endophytic fungi in twigs of healthy and diseased Norway spruce and white fir. *Mycological Res*. 92, 322-326.
29. Somogyi M (1945) A new reagent for determination of sugars. *J. Biol. Chem*. 160, 61-68.
30. Tester M and Davenport R (2003) Na^+ transport in higher plants. *Ann. Bot*. 91, 503-527.
31. Wang Y and Nil N (2000) Changes in chlorophyll, ribulose biphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in Amaranthus tricolor leaves during salt stress. *J. Hort. Sci. Biotech*. 75, 623-627.
32. Yeo AR, Capron SJM and Flowers TJ (1985) The effect of salinity upon photosynthesis in rice (*Oryza sativa* L.): Gas exchange by individual leaves relation to their salt content. *J. Exp. Bot*. 36, 1240-1248.